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Summary

Although some clinicians still believe that cystic fibrosis (CF) lung disease is largely due to hypersecretion of very viscous mucus, it has never been demonstrated that there is mucin hypersecretion in CF and it is clear that there is almost no intact mucin (the principal polymeric component of normal mucus) in CF sputum. CF sputum has lower viscosity when compared to asthma or bronchitis sputa, but is highly tenacious and biochemically most closely resembles pus. Tenacity and lower viscosity lead to decreased cough clearance of infected phlegm, which is thought to induce a persistent inflammatory state in the airway, leading to bronchiectasis. There are many medications and devices either in use or under development that are meant to improve airway hygiene in CF by assisting with sputum expectoration. This paper discusses the scientific basis and potential mechanism of action for many of these interventions and briefly reviews the clinical evidence of their safety and effectiveness. Key words: cystic fibrosis, mucus, phlegm, sputum. [Respir Care 2009;54(6):726–732. © 2009 Daedalus Enterprises]
MUCUS, PHLEGM, AND SPUTUM IN CYSTIC FIBROSIS

The term that we use for the purulent secretion that is a product of airway inflammation is phlegm, from the Greek term for inflammation. This contains breakdown products of inflammatory cells and epithelial cells, including co-polymers of DNA and filamentous (F-) actin, bacteria, cell debris, and variable amounts of mucin.9,10 When phlegm is expectorated, this substance is called sputum. Most studies of airway secretions in people with lung disease have been done using expectorated sputum because of the relative ease of obtaining specimens, compared to the difficulty in obtaining normal mucus from healthy airways.

Phlegm and the Pathogenesis of Cystic Fibrosis Airway Disease

In the previous issue of Respiratory Care, Ratjen discussed the pathogenesis of CF lung disease in detail.11 The normal CF transmembrane ion regulator protein, CFTR (cystic fibrosis transmembrane regulator), maintains adequate airway surface liquid (ASL) volume by net sodium absorption coupled with chloride and water secretion. This periciliary fluid stands about the height of an extended cilium (roughly 7 μm in the trachea and large airways), allowing cilia to extend, and as the ciliary tips interact with the mucus layer, they engage with the mucus. There is biophysical and histological evidence that, in health, a surfactant layer separates cilia from the mucus layer (see Fig. 2), permitting efficient energy transfer without entanglement.12,13

The currently accepted explanation for some aspects of CF airway disease is called the low-volume hypothesis. In this hypothesis, defective chloride secretion and increased sodium absorption in the CF airway leads to an isotonic but low volume of ASL, and this impedes effective mucociliary clearance.14 Cultures of human airway epithelium grown at an air-liquid interface show that CF epithelia have ASL volume depletion (Figure 2).15 It is thought that this leads to mucus adhesion to the airway surface, with plaque formation and entrapment of bacteria causing chronic infection and inflammation and the development of bronchiectasis. This does not explain why there is usually less lung disease in persons who have primary ciliary dyskinesia. Ratjen reviewed the development and testing of ion-transport modifiers to improve CFTR protein channel function or allow alternative channels to reestablish the height of the periciliary fluid by increasing water secretion into the airway.11

It has also been hypothesized that the inhalation of hyperosmolar saline16 or mannitol17 draws water onto the

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Table 1. Sputum Viscosity*

<table>
<thead>
<tr>
<th></th>
<th>Mucoid</th>
<th>Mucopurulent</th>
<th>Purulent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic bronchitis</td>
<td>0.41</td>
<td>0.61</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>(0.08–1.25)</td>
<td>(0.15–1.63)</td>
<td>(0.46–1.38)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>ND</td>
<td>0.39</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.07–0.89)</td>
<td>(0.21–1.63)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>0.25</td>
<td>0.39</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>(0.04–0.46)</td>
<td>(0.16–0.84)</td>
<td>(0.39–1.60)</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.56</td>
<td>1.46</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(0.09–1.72)</td>
<td>(0.26–3.10)</td>
<td></td>
</tr>
</tbody>
</table>

* Expectorated sputa from subjects with chronic bronchitis, cystic fibrosis (CF), non-CF bronchiectasis, and asthma was collected and visually categorized by the degree of "purulence." Viscosity was measured by a rotational viscometer at a single frequency. Although sputa from subjects with all diseases became more viscous with increasing purulence, CF sputum was less viscous than either asthma or bronchitis sputum with similar degrees of purulence. ND = no data available. (Data from Reference 1.)
airway surface, reestablishing ASL volume. However, if this were the principal mechanism of action of hyperosmolar aerosol in CF, the combination of a hyperosmolar aerosol with amiloride to block water and sodium absorption should further hydrate the ASL. Clinically, amiloride does not further improve mucociliary clearance in CF, and actually produces less benefit than the hyperosmolar saline alone.18

Another hypothesis to explain chronic airway infection in CF is that absence of intact airway mucin leaves the epithelium vulnerable to bacterial infection and may make it easier for bacteria to adhere directly to the airway epithelium and to communicate, thus enabling a change in bacteria from free-living or planktonic to the more persistent biofilm morphology.19 This suggests that reestablishment of a protective mucus layer could be important for epithelial defense.

In response to a pulmonary exacerbation of CF the concentration of mucins increases in sputum, suggesting that mucin secretion can be augmented at times of increased infection and inflammation.20 It is likely that mucus secretion is preserved in CF, at least in part, but there is protease degradation breakdown of this defensive layer. It has also been shown that hyperosmolar saline and mannitol are mucin secretagogues in the healthy airway, and this effect is dose-dependent.21 These drugs may play an important role in maintaining airway health, by augmenting mucin secretion.

DNA and F-Actin Polymers in Cystic Fibrosis Sputum

CF sputum polymers are almost entirely neutrophil-derived DNA and F-actin, and these determine the rheologic properties of the sputum.9,10 Under normal conditions such as pneumonia in an immunocompetent host, neutrophils are recruited to the site of infection, and as the infection resolves, the inflammatory cells undergo apoptosis or programmed cell death, with degradation of DNA and F-actin. However, in the CF airway, with greatly enhanced chronic inflammation, neutrophils undergo a necrotic death and DNA and F-actin copolymers form.22 These co-polymers also act as substrates used for the formation of bacterial biofilms.23,24
This secondary polymer network also impedes the passage of nanoparticles, like large proteins, and is a barrier to effective gene therapy.\textsuperscript{25,26} When directly mixed with CF sputum, dornase alfa moderately facilitated the transport of nanoparticles through CF sputum.\textsuperscript{27}

### The Role of Mucolytics in the Treatment of Cystic Fibrosis

#### Classic Mucolytics

By definition, mucolytics are medications that decrease the viscosity of mucus.\textsuperscript{28,29} These have been categorized as classic mucolytics or peptide mucolytics. Classic mucolytics break down mucin oligomers into smaller fragments by severing disulfide bonds that connect mucin monomers across terminal cysteine residues. N-acetyl-L-cysteine (NAC) is the prototype classic mucolytic, but there are other thiol agents that have also been studied for potential mucolytic activity.\textsuperscript{30} None have been shown to be effective as a mucolytic when inhaled as an aerosol, and there is no proven clinical benefit to their use.\textsuperscript{31} Inhaled NAC is very irritating, with pH 2.2. Thus the primary “benefit” of inhaled NAC appears to be the induction of coughing, but at a cost of airway irritation. If intact mucin secretion is important for airway defense, inhaled NAC may be harmful to the epithelium.

NAC is a precursor to glutathione and is an effective antioxidant. There are preliminary reports that high-dose oral NAC may reduce airway inflammation and improve lung health in CF, not as a mucolytic but rather as an antioxidant.\textsuperscript{32}

#### Peptide Mucolytics

Peptide mucolytics break down the secondary polymer network of highly polymerized DNA and F-actin. This polymer network is characteristic of pus. More than a decade ago, dornase alfa (Pulmozyme, Genentech, South San Francisco, California) was introduced as the first peptide mucolytic aerosol for the treatment of CF lung disease. The daily use of dornase alfa significantly improves pulmonary function, and decreases the relative risk of respiratory tract infections in patients with CF lung disease, regardless of the severity of disease.\textsuperscript{33,34} Dornase alfa is administered by jet nebulization at a dose of 2.5 mg once daily. It can be administered before or during the use of airway-clearance devices.

The purulent secondary network also contains substantial amounts of F-actin. This can be depolymerized by actin sequestering and depolymerizing peptides such as thymosin β4.\textsuperscript{35} Thymosin β4 is the major actin sequestering peptide in prokaryotic cells. This drug is currently under development as an aerosol therapy for CF lung disease. It depolymerizes F-actin in CF sputum in vitro, decreasing sputum cohesivity and improving sputum cough clearability. Thymosin β4 also synergizes with dornase alfa so that smaller concentrations of the two mechanisms in vitro decrease sputum viscosity far more than either medication used alone. DNA/F-actin impedes sputum cough clearability in vitro, and changes in sputum DNA concentration over time inversely correlate with changes in cough transportability.\textsuperscript{9}

#### Hyperosmolar Aerosols

Hyperosmolar medications, such as hypertonic saline and mannitol, have been called mucolytic agents, but they do not meet the true definition of a mucolytic because their effect on mucus viscoelasticity is minimal.\textsuperscript{36} Studies by Suri and colleagues suggest that hypertonic saline is not as effective as dornase alfa in improving pulmonary function in persons with CF.\textsuperscript{37} Mannitol may be more appealing to patients, as this is administered as a dry-powder inhaler.\textsuperscript{17}

#### Medications That Promote Sputum Expectoration

Mucokinetic agents improve the effectiveness of cough clearance. Although CF sputum is not particularly viscus, it is extremely tenacious when compared to sputum from patients with other lung diseases\textsuperscript{38} (Fig. 3). There is a surfactant layer in the healthy airway that is thought to be produced by alveolar Type 2 cells or by serous cells in the submucosal glands.\textsuperscript{12,39} Because of this, the surface tension in the normal airway surface is approximately 32 dyn/cm, far less than the surface tension of 71 dyn/cm in the absence of any surfactant.\textsuperscript{13} This surfactant layer is thought to facilitate mucus spreading after secretion from glands and allows efficient energy transfer from cilia to mucus.\textsuperscript{40}
Breakdown of surfactant phospholipids in the CF airway leads to the production of non-surface-active lysophospholipids that increase sputum tenacity, dramatically decreasing the cough clearability of sputum. Surfactant administered as an aerosol for 2 weeks for patients with chronic obstructive pulmonary disease significantly improved pulmonary function and decreased gas trapping. Similarly, phospholipid surfactant liposomes significantly improved the clearability of CF sputum in vitro. Surfactant aerosol is under investigation as a mucokinetic medication for the therapy of CF.

### Decreasing the Volume of Airway Secretions: Mucoregulatory Drugs

Mucoregulatory medications decrease mucin secretion or impede the formation of the DNA/F-actin secondary polymer network. Anticholinergic medications decrease the volume of mucin secreted in response to inflammatory stimuli in experimental animals and in subjects with chronic bronchitis. Although they can produce dry mouth as a result of blocking stimulated secretion of saliva, these drugs do not appear to increase the viscosity of airway mucus.

The most thoroughly studied of the mucoregulatory medications are the low-dose macrolide antibiotics. Azithromycin and clarithromycin decrease interleukin 8 secretion from human airway cells in a non-linear modulation of the hyper-inflammatory response. The mechanism of action appears to be through the modulation of the extracellular regulated kinase (ERK)1/2 cascade. Clarithromycin decreases sputum volume resulting from both inflammation and from mucus hypersecretion in patients with diffuse panbronchiolitis, sino-bronchial syndrome, and non-CF bronchiectasis. Azithromycin improves pulmonary function and decreases the risk of respiratory-tract infections in persons with CF, and macrolide therapy using azithromycin or clarithromycin is now considered part of the standard of care for CF.

### Evaluating the Effectiveness of Interventions to Improve Sputum Clearance in Cystic Fibrosis

In 2007 Respiratory Care published proceedings from a journal conference with the theme of mucus secretion and clearance. At that conference I presented a paper discussing the appropriate design of clinical trials to evaluate mucus-clearance therapy. This information was largely derived from CF research and is summarized here. Sputum color, sputum volume, and perception of sputum thickness are not useful outcomes to evaluate the effectiveness of mucoactive therapy because changes in these do not correlate with any usual measure of any clinical improvement, including pulmonary function, frequency of infection, use of antibiotics, or quality of life. Sputum rheology or viscoelasticity is useful as an in vitro method to determine a range of medication doses that may be effective in vivo. Also, changes in sputum rheology (or lack of changes) can provide information about the mechanism of action of mucoactive medications. However, viscoelasticity of sputum is too variable a measurement to determine if a medication is effective in an individual patient. Furthermore, properties measured in expectorated sputum may not be indicative of these same properties in retained airway phlegm.

Pulmonary function is also easy to measure, and when mucus clearance is improved, there can be significant increases in forced expiratory volume in the first second (FEV). However, FEV is insensitive to all but major changes in the burden of phlegm. It is likely that measurements of gas trapping are more accurate to evaluate the effect of improved or impaired mucus clearance. It is possible that exercise testing, measured by a 6-minute walk test or by shuttle walking, is a useful way to evaluate the effectiveness of mucoactive therapy in CF, if this opens airways and reduces gas trapping.

### Summary

Although CF has been thought to be due to hypersecretion of excessively viscous mucus, we know that this is not the case. In established CF lung disease, the airways are full of phlegm that is similar to pus and contains almost no intact mucin. Therefore, medications aimed at reducing mucin secretion or severing mucin polymers may be of no value and perhaps even be dangerous in CF. Medications meant to decrease the tenacity of sputum containing DNA/F-actin co-polymers are more likely to be of benefit in CF. Because the phlegm tenaciously sticks to the airway epithelium, medications that unstick secretions from the epithelium, such as surfactant, or drugs that promote water secretion into the airway should also benefit patients. The development of techniques to measure the total burden of airway phlegm will help us to better understand the pathogenesis of CF and will be valuable for evaluating therapies meant to promote sputum clearance.

### REFERENCES

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Discussion

Geller: How should a respiratory therapist explain the goals to the CF patient? What are we doing to their mucus with these drugs? We have to put it in words they can understand.

Rubin: Easy. The phlegm in your lungs is not good for you. It’s blocking your airways and it makes you feel bad. It includes bacteria, inflammatory products, all of which contribute to damage your lungs. We know that the more of this stuff we get out, the better you’ll be able to breathe, and the less will be down there to damage your airways. Your body is always producing more, so it’s important that we do these airway clearance maneuvers and take these medications to always keep them clear. You’ve got to stay on top of it, because the sputum is going to keep having to be coughed up, and as long as you keep on top of it and keep them clear, there’ll be less there and it will be better for your lung health.

Rosenblatt: So if a patient tells me they are coughing up more sputum—and I get worried if they tell me that—or if they tell me they feel like they are more congested and can’t get the sputum out, that means nothing?

Rubin: No, sorry; what I meant is that these are not good outcomes for evaluating a therapy. The therapy can cause them to cough up a lot more and they’ll feel better, it’ll cause them to reduce the volume so they can swallow more. But if a patient is coming in on their baseline therapy and saying, “I’m coughing up more, it’s greener, I’m feeling sicker, it’s harder to cough it out,” that usually goes along with all the other signs of what we call a pulmonary exacerbation: decreased pulmonary function, often weight loss, feeling puny. Those are very good signs associated with an exacerbation in somebody at their baseline. I wouldn’t use those, though, for an outcome to see if they’re getting any improvement with a new therapy.

Rosenblatt: If they come in with those findings that indicate they’re having an exacerbation, but then they go back to their baseline, we can’t say that they are getting any better?

Rubin: No, I would. But if you have somebody coming in and you’re initiating dornase therapy (Pulmozyme) or you’re teaching them to do active cycle breathing, for example, and you say, “I want to see how this is going to work for you and what we’re going to do is measure how much sputum you can cough up each morning, or look at the color of the sputum,” that really doesn’t give you any indication if the therapy is working. Studies that have used those, where they’ve quantified the volume of secretion, or looked at the color or texture, haven’t correlated at all with how the patient feels, respiratory-tract exacerbations, use of antibiotics, or pulmonary function. So, although those are useful in a patient when they’re feeling bad, and are decent clinical symptoms that go along with an exacerbation, they aren’t useful outcome measurements for determining if an intervention is of benefit.

Rosenblatt: So they are good clinical markers at the bedside to determine how well a patient is doing, but they should not be used as markers in an objective study.

Rubin: Sure. If you put somebody on a vest, which we know is an effective method of airway clearance, and they say, “I’m coughing up a lot more,” the next questions should be, “Well, how do you feel? What can you do? What is your energy?” Some of these new things that we’re bringing on—hyperosmolar solutions are an example—they’re mucus secretagogues; they’ve been shown to induce secretion of mucus, of true mucus with mucins. If somebody is expectorating a lot more, you don’t know if it’s a good thing, if it’s a bad thing, or if it’s neutral. So it’s not the volume or color of what you’re coughing up as a response to therapy, but what you can cough up and how you feel do tend to go hand in hand on your baseline. Is that a little bit more clear? It’s not useful as an outcome for therapeutic interventions, but it appears to be useful as a clinical sign of deterioration or a recovery.

Rosenblatt: I’m a little viscous, so it’s OK.